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PB-278 512

Evaluation and Research of Methodology for the National Environmental Specimen Bank

National Bureau of Standards, Washington, D.C.

Prepared for

Health Effects Research Lab, Research Triangle Park, N C

Feb 78

PB 278 512

EPA-600/1-78-015
February 1978

Environmental Health Effects Research Series

EVALUATION AND RESEARCH OF METHODOLOGY FOR THE NATIONAL ENVIRONMENTAL SPECIMEN BANK

Health Effects Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina 27711

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TECHNICAL REPORT DATA
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1. REPORT NO. EPA-600/1-78-015	2.
3. RECIPIENT'S ACCESSION NO. PB278512	
4. TITLE AND SUBTITLE: Evaluation and Research of Methodology for the National Environmental Specimen Bank	5. REPORT DATE: February 1978
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S): T.E. Gills, H.L. Rook and P.D. LaFleur, Editors	8. PERFORMING ORGANIZATION REPORT NO
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Institute for Materials Research, National Bureau of Standards, Washington, D.C. 20234	10. PROGRAM ELEMENT NO. 1AA601
	11. CONTRACT/GRANT NO. EPA-IAG-D5-0568
12. SPONSORING AGENCY NAME AND ADDRESS: Health Effects Research Laboratory RTP,NC, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, N.C. 27711	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES

16. ABSTRACT

This report is a summary of analytical methodologies developed or adopted by NBS to insure proper procedures for sampling, storage and analysis of biological and environmental type matrices. Protocols established in these procedures will insure that the measurement of an analyte can be made within known and/or required uncertainty levels and that samples can be stored for retrospective analysis.

The contributions in this report are divided in seven sections which includes analytical methods for trace element analysis and techniques for sampling and storage of specific matrices.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
standards chemical analysis biological extracts	specimen bank	07 C 05 B 06 F

18. DISTRIBUTION STATEMENT: RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES 78
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE PC A05 / MF A01

EPA Form 2220-1 (9-73)

EPA-600/1-78-015
February 1978

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory develops and revises air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is preparing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

This report documents one aspect of an International effort, supported by EPA, to provide a comprehensive environmental monitoring program to assess the relative risk of environmental hazard to the health and well-being of our population and to aid in the improvement of our environmental quality. This program, the National Environmental Specimen Bank, will serve as an environmental warning system by providing real time chemical analysis of collected specimens. In addition, this system would permit the use of tomorrow's more sensitive and more specific methods of chemical analysis on stored samples. The advantages of such a program will permit us to assess the effectiveness of our present environmental control techniques by monitoring pollutant trends, as well as establishing environmental baseline levels of new pollutants or pollutants of current concern not previously investigated.

John H. Knelson, M.D.
Director,
Health Effects Research Laboratory

ABSTRACT

This report is a summary of analytical methodologies developed or adopted by NBS to insure proper procedures for sampling, storage and analysis of biological and environmental type matrices. Protocols established in these procedures will insure that the measurement of an analyte can be made within known and/or required uncertainty levels and that samples can be stored for retrospective analysis.

The contributions in this report are divided in seven sections which includes analytical methods for trace element analysis and techniques for sampling and storage of specific matrices. This report is submitted in partial fulfillment of EPA Interagency Agreement IAG-D5-0568 by the National Bureau of Standards. This is the final report for the 1976 contract year.

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ACKNOWLEDGMENTS

The authors of this report wish to acknowledge their appreciation to the Environmental Protection Agency for their funding. We especially want to thank Dr. George Goldstein, Project Officer of the Clinical Pathology Branch for his consultations and program guidance. Finally, we extend sincere thanks to the operating staff of the NBS Research Reactor and the NBS Electron Linac Facility for the fine service provided.

SECTION 1

THE DETERMINATION OF IODINE IN BIOLOGICAL AND ENVIRONMENTAL STANDARD REFERENCE MATERIALS

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INTRODUCTION

The accurate determination of iodine in biological samples has been of interest in many medical and nutritional studies. The importance of iodine to thyroid function is a well understood biochemical relationship; however, the role of iodine in other biological functions is not well understood. One of the main obstacles to this understanding has been the inability to measure accurately iodine at the concentration levels needed. Exclusive of thyroid tissue, iodine levels in human soft tissue and serum are in the range of 0.0001 to 0.1 μg iodine per gram wet tissue.

Few analytical techniques have the demonstrated sensitivity or accuracy for the determination of iodine at this level. Thermal neutron activation analysis is one technique that does have the required inherent sensitivity. The combination of a large thermal neutron cross section and a relatively short half life for the product iodine-128 would normally yield more than adequate sensitivity. However, in biological systems, one complicating fact has confounded analytical chemists for years. The very high levels of chlorine, and to a lesser extent bromine, coupled with the similar chemistry of these elements has made the practical limit of detection much higher than the theoretical limit.

The determination of iodine in biological material by neutron activation analysis has received considerable attention. It was observed that nondestructive techniques were inadequate except in samples with unusually high levels of iodine such as thyroid tissue or kelp (1). During this period, numerous publications appeared detailing radiochemical separations for iodine. Most, however, were of a similar procedural nature. Samples were rapidly dissolved, iodine carrier added, and the iodine separated by either liquid-liquid extraction or by liquid-ion exchange (2,3). Methods have been reported where the iodine was distilled directly from the matrix (4,5) but even in these procedures, a subsequent separation was performed to obtain the necessary decontamination from chlorine.

The best indication of the state of iodine analysis in biological matrices is the limited number of publications containing data on iodine analyses. A review of published iodine values on Bowen's kale indicated a limited data set with a variability so large as to invalidate any consensus mean value (6). To the author's knowledge, no values have been reported for iodine on either NBS SRM's 1571, Orchard Leaves, or 1577, Bovine Liver. A recent publication on environmental matrices reported

iodine results for NBS SRM 1632, coal. These results were achieved using instrumental neutron activation in a matrix where chlorine was not a severe problem and were at a significantly higher iodine concentration than normally found in biological samples (7). In spite of the favorable analytical conditions, these results appear to be high when compared to those obtained in this work.

This paper presents a new method for the determination of iodine in biological matrices using a unique adaption of the general gas phase separation of volatile elements as developed at NBS (8,9,10). The procedure involves controlled combustion of the sample followed by direct gas phase separation of the chlorine and bromine from iodine using hydrated manganese dioxide. This procedure has four major advantages over conventional chlorine/iodine separations.

- The procedure is rapid, requiring less than one half-life of iodine-128 for complete separation.
- The procedure is simple, as it involves no extractions or other complex chemical manipulations, thereby minimizing technique related errors.
- The separation is quantitative, thereby eliminating the need for chemical yield determinations and the associated additional errors.
- The procedure gives better than a 10^5 to 1 separation of chlorine from iodine. This is far superior to liquidextraction procedures and essentially eliminates the Compton baseline from chlorine-38.

The accuracy of the described analytical procedure has been verified by independent photon activation analysis on selected samples.

APPARATUS AND REAGENTS

COMBUSTION TUBE

The combustion tube consists of a Vycor tube, 50 cm long with a 19/38 standard taper joint at the downstream end. The inner portion of the standard taper joint contains a 5 cm long column of hydrated manganese dioxide inorganic ion exchange material. The downstream portion of this column has an outer 5/12 standard taper joint welded to it. The inner portion of the 5/12 joint contains silver coated glass wool which is used to trap all remaining halides. The entire combustion tube and gas phase separation assembly is shown in figure 1.

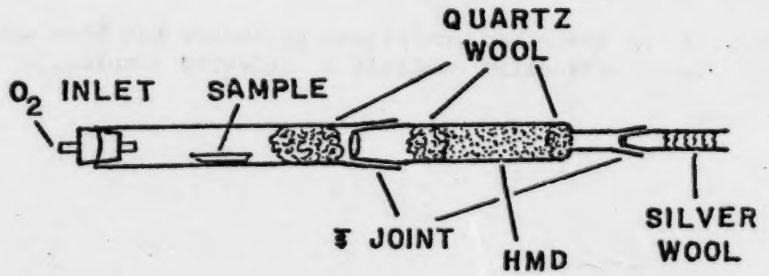

Figure 1: Combustion Apparatus

IRRADIATION SOURCE

All samples were irradiated in the pneumatic transfer tube RT-3 of the NBS research reactor. The thermal neutron flux was approximately 6×10^{13} $n \cdot cm^{-2}$ sec^{-1} and the Cu/Cd ratio was $\sim$100.

COUNTING EQUIPMENT

All samples were counted on a 25 cm^3 volume Ge(Li) detector with a 2.1 keV resolution for the 1332 keV photo peak of ^{60}Co. The detector was coupled to a Hewlett Packard Computer-Analyzer.* Quantitation of the individual photopeaks was performed directly using the total peak area method.

GAS PHASE CHLORINE TRAP

The gas phase chlorine trap consisted of a column of Carlo Erba manufactured hydrated manganese dioxide (HMD). The trap had a minimum dimension of 5 cm length and 0.8 cm diameter. The ion exchange material was held in place by two plugs of quartz wool. Columns were prepared by simply pouring the material, as received, into the column with very gentle side tapping for packing. The column was capable of passing a gas flow of 100 cm^3 per minute with negligible build up of pressure.

IODINE TRAP

The glass wool was coated with a heavy silver deposit. Any conventional silvering process is adequate. After silvering, the material was washed with three successive portions of distilled water and oven dried. The silver wool was packed tightly into a column 4 cm long and 0.5 cm in diameter. The portion of the trap utilized for iodine collection is clearly visible due to the formation of yellow silver iodide. Trap lengths should be adjusted to accommodate the quantity of iodine carrier used.

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*Certain commercial equipment, instruments, or materials are identified in this paper in order to adequately specify the experimental procedure. In no case does such identification imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the material or equipment identified is necessarily the best available for the purpose.

EXPERIMENTAL

PROCEDURES

Samples of biological materials were weighed, as received, into precleaned quartz tubes. Sample weights of 200-500 mg were used for analysis. Replicate samples of each material were vacuum dried for 24 hours to obtain dry weight values. Standard solutions of 250 and 500 ml were also weighed and sealed in quartz tubes. Individual samples were irradiated for 15 seconds, separated, and counted for two successive 10 minute counts. Standards were processed in the same manner as the samples and were run a minimum of one per every five samples. All analysis sets were begun and finished with a standard sample.

Following irradiation, the quartz vials were washed in 6 M HCl, rinsed with water and wiped dry. The samples were then cooled in liquid nitrogen and opened. The bulk material was transferred to a combustion boat and 100 µl of iodine carrier solution containing 2 mg of KI was added. The combustion boat plus quartz irradiation vial were inserted into the combustion tube and an oxygen flow of ∿100 cc/min was passed over the sample. The sample was ignited by heating the combustion tube directly over the sample with an oxygen-gas torch. Most biological samples ignited readily as the quartz tube reached a dull white heat. Once ignited, the samples were allowed to burn freely with the intensity of the burn controlled by the oxygen flow. During the combustion process, the entire assembly was heated moderately with the gas torch to prevent the condensation of water vapor. When the self-maintained combustion ceased, the sample was heated to a dull red heat with the gas torch to insure complete combustion. Then, a resistance furnace was placed over the entire combustion tube and the sample heated to 1000 °C to drive all of the iodine through the trap and onto the silver wool collector. The collector was also heated gently to drive off all residual condensed water. Care must be used in this drying step as silver iodide is volatized at slightly over 500 °C.

Following distillation, the silver wool collector was removed, sealed in a polyethylene bag and placed 5.0 cm from the detector. Two successive ten minute counts were taken to check the half life of the iodine-128. The 441 keV photopeak of iodine-128 was used for analysis. When sufficient iodine-128 activity was ovtained, the 528 keV photopeak was also quantified as a validation. Standard solutions were processed and counted with exactly the same procedure as the samples. Quantitative results were obtained by the comparator method.

RESULTS AND DISCUSSION

Gas phase separations to isolate selectively radiochemical species have been used successfully in activation analysis. This type of separation has been applied most successfully for the determination of mercury in complex matrices (8). More recent work applied the technique to the separation of selenium and to a group separation of Hg, Se, Zn, As, and Ce (9,10). All of the methods had one underlying principle in common, however, that the isolation of the total volatile component of the sample was sufficient separation for elemental identification and quantitation. One method for the determination of mercury developed by Kosta, *et al.*, introduced a subtle new concept (11). In this method, a silver wool adsorption trap, heated to 300 °C, was placed in the gas stream to remove halides while allowing mercury to pass to a final collector. This process of selective gas phase adsorption offers one of the most rapid, simple procedures for radiochemical separations.

In 1974, work at NBS was begun on the analysis of iodine by gas phase distillation. Iodine had previously been observed to distill quantitatively in an oxygen atmosphere. This procedure was marginally attractive for longer half life iodine isotopes such as iodine-131 and iodine-133, but in almost all cases, the predominance of chlorine-38, which codistilled with the iodine, precluded the direct analysis of total iodine. Attempts to separate the halides by conventional wet chemical procedures, after distillation, did not prove satisfactory. Separation efficiencies were generally not high enough to remove sufficient chlorine in one step and the additional time required severely degraded the sensitivity of the technique.

In an attempt to accomplish the entire separation in the gas phase, various inorganic ion exchange compounds were studied as selective adsorption traps. These compounds were attractive in their ability to withstand high temperature gas streams. To facilitate the early work, the longer half life iodine species were studied. It was found in this study that HMD, under carefully controlled temperature conditions, exhibited selective adsorptive properties for bromine while quantitatively passing iodine. The culmination of this work was a procedure for the determination of tellurium and uranium using their iodine daughters (12).

Following this work, the use of HMD to separate chlorine from iodine was studied. Initial separations using iodine-126 and chlorine-38 traces indicated that HMD was a superior material for the selective adsorption of

chlorine. To verify this, one hundred microliters of standard solution of each isotope were pipetted onto 250 mg of nonradioactive orchard leaves. The orchard leaf was burned and the gaseous products were distilled through a warm HMD trap and collected quantitatively on the silver collector. No evidence of chlorine-38 activity was observed on the silver collector. Separation efficiencies of greater than 10^4 were estimated and a series of these experiments indicated respectable collection efficiencies of greater than 95 percent for iodine (Table 1).

TABLE 1. PERCENT RECOVERY FOR IODINE/CHLORINE SEPARATED USING HMD

Run	% Recovered ^{126}I	Separation Efficiency for $^{38}Cl^{\dagger}$
1	97.4	$> 10^3$
2	99.0	$> 10^3$
3	96.3	$> 5 \times 10^3$
4	98.8	10^4
5	98.2	2×10^4
Mean	97.8	

†Separation efficiency for $^{38}Cl = \frac{^{\circ}A\ ^{38}Cl \text{ on HMD}}{^{\circ}A\ ^{38}Cl \text{ on Silver}}$

After this initial work, samples of orchard leaves and bovine liver were irradiated for 15 seconds. Aliquots of the standard iodine-126 solution were added, and the iodine was separated. Again the iodine-126 was recovered quantitatively, and iodine-128, induced from the iodine in the sample, was observed. In the case of bovine liver, chlorine decontamination of 10^5 was obtained.

With the separation satisfactory in every respect, a detailed procedure for the analysis of total iodine was devised. This procedure is given in the Experimental section. Replicate samples of NBS SRM's 1571, 1577, and 1632, orchard leaves, bovine liver, and coal, were analyzed using this procedure. In all cases, the iodine-128 was easily observed after the separation. Freshly prepared standard solutions, made from high purity potassium iodide and cesium iodide were used as primary standards. These solutions were carried through the same procedure as the samples. Results of these analyses are given in Table 2.

TABLE 2. CONCENTRATION OF IODINE IN STANDARD REFERENCE MATERIALS

Sample	Iodine Concentration in μg/g Orchard Leaves SRM 1571	Bovine Liver SRM 1577	Coal SRM 1632
1	0.176	0.187	2.52
2	0.165	0.176	3.01
3	0.171	0.208	2.54
4	0.180	0.199	2.60
5	0.160	0.173	2.71
6	0.166	0.181	
7	0.153	0.183	
Mean	0.16_7	0.18_0	2.6_8
†s	0.01	0.01_2	0.2_0

†s is the standard deviation of the mean.

As a final verification of the method, samples of these materials were independently determined by nondestructive photon activation analysis (13). A summary of these results is given in Table 3. The agreement among results is relatively good in all cases, but the errors were large in the cases of orchard leaves and bovine liver as the photon method was near its limit of sensitivity.

TABLE 3. COMPARATIVE RESULTS ON IODINE IN STANDARD REFERENCE MATERIALS

Material	This Work	Photon Activation Analyses[1]
SRM 1571 Orchard Leaves	0.17±0.01	.10 ± 0.05
SRM 1632 Coal	2.7 ±0.2	3.3 ± 0.4

[1] Results obtained in private communication from Dr. John Hislop, Environmental and Medical Sciences Division, Harwell, England.

In conclusion, the gas phase separation of iodine using combustion and selective adsorption appears to be a simple, attractive method for the analysis of total iodine in complex matrices. Separations can be completed in 10-15 minutes with a total analysis time of less than 60 minutes. With this procedure, accurate iodine analyses can be performed in samples which were heretofore difficult or impossible to analyze.

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SECTION 2

DETERMINATION OF CHROMIUM IN BIOLOGICAL MATRICES BY NEUTRON ACTIVATION ANALYSIS: APPLICATION TO STANDARD REFERENCE MATERIALS

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INTRODUCTION

Chromium has been recognized as an essential trace element (1) in human nutrition for several years. At high concentrations or in different chemical states it can also cause deleterious or toxic effects. There is a need for reliable methods of analysis for chromium at trace levels in a variety of matrices, especially biological matrices. A laboratory intercomparison on environmental materials (2) pointed up the sad state of chromium determinations as performed by most of the analytical community. Also, the author has observed a wide range of results reported for chromium in the National Bureau of Standards (NBS) Bovine Liver Standard Reference Material (SRM 1577). As a result of these observations and communication with several analytical laboratories involved in the determination of chromium in biological matrices, persons at NBS became convinced that more biological materials needed to be analyzed and certified for chromium content.

Neutron activation analysis, both instrumental (INAA) and destructive (DNAA), has been used to determine the chromium concentration in a variety of matrices. The results obtained for chromium in two biological Standard Reference Materials (SRM's)--Orchard Leaves, and Brewers Yeast--are described in this paper. The chromium content has been certified by NBS in these two SRM's.

EXPERIMENTAL

Samples Analyzed

The biological materials analyzed were NBS Standard Reference Materials Orchard Leaves (SRM 1571), and Brewers Yeast, which was furnished to NBS by the Nutrition Institute, U.S. Department of Agriculture, Beltsville, Maryland, for preparation as a Standard Reference Material. The Brewers Yeast is certified for total chromium concentration only and is currently being prepared for sale by the NBS Office of Standard Reference Materials as SRM 1569.

Preparation of Standards and Carrier

Two standards were used in this work. One standard was prepared by dissolving a weighed amount of chromium metal (99.99% pure) in high purity HCl and diluting to a specific volume with high purity H_2O to obtain the desired Cr concentration. The second standard was prepared by dissolving a weighed amount of NBS-SRM 136C ($K_2Cr_2O_7$) in high purity H_2O and diluting to volume to obtain the desired Cr concentration. Chromium carrier solutions were prepared from analytical reagent grade $CrCl_3$ and $K_2Cr_2O_7$ and normally

contained 3 mg Cr/ml.

All reagents used in the analysis were of analytical grade, unless stated otherwise.

Irradiation Conditions

Chromium was determined in the two biological materials using both INAA and DNAA. The samples (200-350 mg) along with Cr standards were encapsulated in clean Supersil quartz vials and irradiated for periods of one to six hours in the NBS Reactor at a thermal neutron flux of $1x1^{13}$n cm^{-2}. sec^{-1}. The samples were allowed to decay 3-6 weeks for INAA work and ∿36 hours for DNAA work to reduce the matrix activity before processing.

Procedure for INAA

Following a three-week decay the sample vials were washed clean of exterior contamination with 1:1 HNO_3 and H_2O, frozen in liquid nitrogen opened, and the material was transferred to clean polyethylene counting vials. The amount of sample transferred was determined by weight. The samples and standards were counted on a 75 cm^3 Ge(Li) detector coupled to a 4096 channel pulse height analyzer for measurement of ^{51}Cr produced by the $^{50}Cr(n,\gamma)$ ^{51}Cr reaction. The concentration of chromium was determined by the direct-comparator method.

Several brewers yeast samples and standards were preweighed into clean quartz vials, sealed, and irradiated as already described. After allowing these samples to decay for ∿6 weeks, the vials were washed clean of exterior contamination and counted (material still contained in vial) as already described.

Procedure for DNAA

Following a 36-hour decay, the sample and standard vials were cleaned, frozen in liquid N_2, opened, and transferred (by weight) to a 50 ml Erlenmeyer flask designed for dissolving materials in a closed system (3) and trapping volatile material in a trap solution. Chromium carried (∿5 mg of both Cr III and Cr VI) and 5-10 ml of concentrated $HClO_4$-HNO_3 mixture (1:3) were added to the flask. The flask was heated on a Pyrex-top hot plate until all the sample had dissolved and all the vapor fumes visibly trapped in a 1:1 HNO_3 solution. The flask was removed from the heat and 5 ml of hot $Ce(SO_4)_2$-3M H_2SO_4 solution (10% w/v) was added; the flask was heated an additional 5-10 minutes to assure oxidation of and to maintain the chromium as Cr VI. After cooling, the sample solution in the flask was transferred to a 50-ml extraction tube with 15 ml of 1.5 M HCl. The sample was then extracted with 10 ml of 1 percent (w/v) tribenzylamine-chloroform solution. A 5-ml aliquot was taken from the organic phase and counted on a 25 cc Ge(Li) detector coupled to a HP 4096 computer-analyzer. The trap solution from the dissolution step was transferred to a suitable counting vessel and counted on this system also. The 320 keV gamma ray peak of ^{51}Cr was integrated and the chromium concentration was determined by the direct-comparator method using the two chromium standards subjected to the same

procedure. All of the chromium from these standards remained in the flask. The trap solution was checked for chromium but none was detected. The procedure is the same for open-flask dissolution of samples except a regular Erlenmeyer flask is used, eliminating the trap solution.

RESULTS AND DISCUSSION

Recent interlaboratory comparison studies (4,5) of analytical methods and their results for the analysis of chromium in biological and environmental materials showed very large differences in analytical values. During the spring of 1974, a workshop on chromium analysis was held at the University of Missouri in Columbia where these differences were discussed and recommendations made to resolve them. There was consensus among the participants that biological standard reference materials, certified for total chromium content by NBS, would provide a starting point for the analytical community to critically evaluate their methods.

Chromium has been determined and certified in several materials by NBS. However, this manuscript describes the results of chromium analyses in two biological materials--Orchard Leaves, Brewers Yeast--using neutron activation analysis (NAA). Studies here in our laboratory and other laboratories (6,7) on some biological materials had suggested possible losses of chromium content during sample manipulation (e.g., dissolution, charring, ashing, etc.). Instrumental neutron activation analysis (INAA) and destructive neutron activation analysis (DNAA) were used to determine the chromium concentration in these biological materials. The use of INAA to determine the total chromium concentration in these biologicals provided a check for the values obtained using DNAA and also other techniques which required sample treatment. The results obtained for chromium in the two biologicals mentioned above, using NAA and the NBS Reactor, are given in Tables 1-4.

By employing two dissolution techniques--closed system and open flask--for the irradiated biologicals, the loss of chromium in the dissolution step could be evaluated. The results obtained for chromium in Orchard Leaves (Table 1) were essentially the same using INAA and the two dissolution techniques, indicating no chromium is lost from this material in the dissolution procedures used.

A large amount of matrix radioactivity is produced when these biological materials are irradiated. Much of this activity results in long-lived beta radiation creating a bremsstrahlung continuum in the gamma spectra of each sample. This continuum affects the low energy range of the spectra where the chromium photopeak is located thus arge background corrections are necessary. Radiochemical separation of Cr (DNAA) eliminates this background interference. The results obtained from open-flask dissolution (DNAA) of yeast (Table 2) were consistenlty lower ($\sim$25%) than those obtained instrumentally (INAA). The results obtained from dissolution of brewers yeast in a closed system as described in the DNAA procedure are in good agreement with the INAA results.

The results in Tables 1-3 show chromium can be lost during sample dissolution of brewers yeast. However, no loss of chromium was observed in our experimental design for the analysis of chromium in orchard leaves. The chromium loss observed in brewers yeast is probably due to a volatile component during the acid attacks. Further studies to characterize this component are underway in this laboratory.

To establish that chromium was not lost in the irradiation step, brewers yeast was preweighed, packaged, irradiated, and counted in the same container. The results in Table 3 show the amount of chromium obtained using a polyethylene irradiation container (INAA-P.E.) are in good agreement with those obtained from a quartz irradiation container (INAA-Quartz).

The radiochemical procedure described for chromium (DNAA) provides the analyst with a simple, rapid, and selective technique for chromium determination in a variety of matrices. The procedure is also adaptable for use by other techniques. Results of chromium analysis (from a variety of techniques) reported in the literature in recent years show very large differences in concentration on the same materials. Thus, it is hoped these two biological materials, certified for total chromium concentration, will help those involved in chromium analysis to evaluate their methods and improve chromium results reported in the literature and elsewhere for analytical quality control samples.

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TABLE 1. CONCENTRATION OF CHROMIUM IN ORCHARD LEAVES, SRM 1571, (μg Cr/gram)

Spl #	INAA	DNAA[A]	DNAA[B]
1	2.590	2.514	2.472
2	2.574	2.489	2.447
3	2.563	2.499	2.484
4	2.567	2.495	2.433
5	2.569	2.501	2.463
6	2.582	2.472	2.481
$\bar{X}$	2.574 ± 0.010	2.495 ± 0.014	2.463 ± 0.020

* NBS Certified Value: 2.6 ± 0.2 μg/g

Note: A - Closed system dissolution

B - Open flask dissolution.

* Based on results of two independent analytical methods--- This work and isotope dilution mass spectrometry.

TABLE 2. CONCENTRAION OF CHROMIUM IN BREWERS YEAST, SRM 1569 (µg Cr/gram)

Spl #	INAA	DNAA[A]	DNAA[B]
1	2.079	2.070	1.583
2	2.075	2.068	1.543
3	2.090	2.062	1.548
4	2.077	2.068	1.588
5	2.067	2.088	1.551
6	2.104	2.090	1.565
$\bar{X}$	2.082 ± 0.013	2.074 ± 0.012	1.558 ± 0.015

NBS Certified value: 2.12 ± 0.05 µg/g --- Based on results of two independent analytical methods--This work and isotope dilution mass spectrometry.

Note: A - Closed system dissolution.

B - Open flask dissolution.

TABLE 3. CONCENTRATION OF CHROMIUM IN BREWERS YEAST, SRM 1569, Using INAA (μg Cr/gram)

Spl #	INAA - P.E.	INAA - Quartz
1	2.079	2.131
2	2.105	2.087
3	2.117	2.137
4	2.081	2.092
5	2.120	2.149
6	2.075	2.120
$\bar{X}$	2.096 ± 0.020	2.119 ± 0.025

SECTION 3

METHOD FOR THE DETERMINATION OF PLATINUM IN BIOMATERIALS

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INTRODUCTION

Heavy metal contamination of the environment has received much attention as serious pollution and health problems. Among the metals, platinum is receiving increasing attention because of its many uses as a catalyst, particularly in the catalytic converters used in the automotive industry. Neutron activation analysis has the sensitivity for determining platinum at very low concentrations, however the most sensitive and useful nuclear reaction involves isotopes of relatively low gamma energies, thus requiring separation of these isotopes from an activated matrix. Similar methods (1,2) have been used for the determination of platinum in glasses and indium and copper in gold.

The technique involves the spontaneous deposition of one metal from solution using a noble metal (i.e., one above it in the electromotive series) in the metallic form. Specifically, ^{199}Au (as the chloro complex) formed from the decay of ^{199}Pt is deposited using silver metal.

The analysis for platinum was made by irradiating samples with neutrons and subsequently separating the element of interest by spontaneous deposition. The nuclear reaction most suitable for the analysis was $^{198}Pt(n,\gamma)^{199}Pt \xrightarrow{\beta} {}^{199}Au$. The end product of the reaction, ^{199}Au has a 3.15 day half-life and γ-ray energies of 158 keV (37% abundant) and 208 keV (8% abundant).

EXPERIMENTAL

The encapsulated samples, along with the platinum standards, were irradiated in the NBS Research Reactor at a thermal neutron flux of $\sim 6 \times 10^{13} n \cdot cm^{-2} \cdot sec^{-1}$ for one hour. After irradiation, the samples were allowed to decay for 24 hours to reduce short half-life matrix activities. The samples were then quantitatively transferred to 100 ml beakers, and carriers of Pt, Na, Ba, and Mn were added. The samples were dissolved in 10 ml of hot aqua regia and reduced in volume, by evaporation, to 2 ml. After cooling, the solutions were washed into 25 ml of water containing 50 mg of finely divided silver powder. These mixtures were shaken, in capped polyethylene bottles, to allow the spontaneous reduction of the platinum. The excess silver metal, containing the platinum, was filtered onto 25 cm glass fiber-filter pads, mounted in 42 cm petri dishes, and counted in a standard geometry.

INSTRUMENTATION

The counting was carried out using a 75 cc Ge(Li) detector coupled to a 4096 Channel Pulse Height Analyzer. Spectra were read onto magnetic tape and processed with the ALLSPICE and QLN1 computer codes. Both the 158 keV and 208 keV gamma peaks of ^{199}Au were quantified.

REAGENTS

The platinum standard used was NBS Standard Reference Material (SRM) 680, High Purity Platinum. The SRM was in the form of 0.0508 cm (.020 inch) diameter wire, and had a purity of 99.998 percent.

All other reagents were of ACS analytical reagent-grade quality.

SAMPLE PREPARATION

Samples were prepared for reactor irradiation by weighing into polyethylene micro vials. Carbonaceous materials such as the filter papers were high-temperature ashed in porcelain crucibles before encapsulation.

Platinum standards were prepared by dissolving a weighed quantity of high purity platinum metal with a minimum volume of hot aqua regia.

RESULTS AND DISCUSSION

Analytical results are given in Table 1. Concentrations were calculated using the standard-comparator method. The concentration specified are given in addition to the concentration found. The overall analytical error is estimated to be ±]0 percent, even though certain individual results may be significantly better. Both photopeaks of ^{199}Au were individually quantified with consistent results in all cases. The absence of ^{199}Au in significant amounts preclude the remote possibility of double neutron capture by large quantities of stable gold, leading to ^{199}Au which was used in the analysis.

TABLE 1. PLATINUM DOPED FE_2O_3 WITH FUSED SILICA

Sample ID	Specified PT µg/g Concentration	Concentration Pt µg/g Found	
2	4	4.4 4.7 3.6 3.8	$\bar{x} = 4.1$
3	2	1.5 1.8	$\bar{x} = 1.6$
4	.5	.42 .42	$\bar{x} = .42$
5	X	$\leq .1$ $\leq .1$	≤ 0.1

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SECTION 4

A METHOD FOR THE DETERMINATION OF ARSENIC, ANTIMONY, AND COPPER IN BIOMATERIALS BY NEUTRON ACTIVATION ANALYSIS

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INTRODUCTION

The environmental impact of metal pollutants that can lead to deleterious effects on human health have been well documented. Because of the severe toxicity of some metals such as arsenic, antimony, selenium, etc., levels of less than 1 ppm must be routinely determined to ensure the safety of both plant and animal life. Neutron activation analysis has the sensitivity for determining many of these toxic elements and is an ideal technique for determining trace metals in biological materials. However, in many situations the most sensitive and useful nuclear reactions involves isotopes of relatively low gamma energies; thus requiring separation of those isotopes from a neutron activated matrix to obtain maximum sensitivity and accuracy.

Among possible reagents for the radiochemical separation of toxic trace metals, inorganic ion exchangers have recently received some attention[1]. By selecting the proper acidic media and inorganic ion exchangers, very selective separations were achieved. A cuprous chloride column in series with a tin dioxide column, equilibrated in a 6M $HClO_4$ media proved effective in isolating ^{64}Cu, ^{122}Sb, ^{76}As, respectively. This separation scheme was tested using radioactive tracers and by reanalyzing NBS Orchard Leaves SRM 1577. The procedure was later applied to the certification of As, Cu, and Sb in the following NBS proposed SRM's: Spinach, Pine Needles, and Tomato Leaves (1573), (1570), and (1575).

This procedure involved the neutron irradiation of a biological sample and subsequent mineralization of the material by nitric and perchloric acids. After obtaining the irradiated material in a soluble form, the solutions were adjusted to the desired acid concentrations and passed through chromatographic columns of cuprous chloride and tin dioxide which had been equilibrated with 6M $HClO_4$. Copper and antimony were sorbed on the cuprous chloride and arsenic sorbed on the tin dioxide. Sodium, the major interference in the analysis of biological materials, was eliminated by eluting with 6M $HClO_4$.

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EXPERIMENTAL

Samples of each material (weighing approximately 250 mg each) were encapsulated in polyethylene microcentrifuge tubes. Solution standards of Cu, As, and Sb, as well as NBS Orchard Leaves SRM #1571, were also encapsulated to quantitate the three botanicals for Cu, As, and Sb. The samples were then irradiated for 30 minutes in RT-3 of the NBS reactor in a flux of approximately 5×10^{13} n $cm^{-2} sec^{-1}$. Because of the high matrix radioactivities, the irradiated materials were allowed to decay for 24 hours to reduce personnel radiation exposure. After decay the samples were post weighed into 125 ml Erlenmeyer flasks containing carriers (21 mg) of each element to be analyzed. The samples were then dissolved in a 1:3 mixture of $HCLO_4$-HNO_3 with 3-4 drops of conc. HF to dissolve any siliceous material. After dissolution the samples were taken to near dryness and allowed to cool. Ten milliliters of 6M $HCLO_4$ were added to each sample and the solution then passed through 3 cm columns of cuprous chloride and tin dioxide. Copper was absorbed on the cuprous chloride and antimony and arsenic were absorbed on the tin dioxide column. The columns were then washed with two ten milliliter portions of 6M $HCLO_4$. After the final wash the columns were emptied separately into 45 cm petri dishes and counted.

INSTRUMENTATION

The counting was done on a 25 cc Ge(Li) detector in conjunction with a 2048 channel pulse height analyzer. The peak analyses were done by a Hewlett Packard analyzer-computer.

REAGENTS

The standard solutions were made of high purity metals or oxide dissolved in NBS high purity acids. All other reagents were of ACS analytical reagent-grade quality.

SAMPLE PREPARATION

Samples of each of the proposed SRM's were freeze-dried at room temperature. The nominal weight loss was experimentally determined to be ±2 percent.

RESULTS AND DISCUSSIONS

The results are given in Table 1. Concentrations were calculated using the standard-comparator method. The described procedure gave precisions acceptable for certification. As to the accuracy of the analysis, a comparison of these results to those obtained by other independent methods is shown in Table 2. The comparison shows relative good agreement with other methods. Inorganic exchangers have proved potentially useful for radiochemical separations. Such separations have the following advantages:

- Effective separations from strong acidic media (> 1 molar) that can be easily adjusted from typical dissolution procedures.
- Quantitative separations, thus eliminating errors involved in chemical yields.
- High decontamination from activated biological matrices.
- Multielement analysis consequently reduced analysis cost

With this procedure rapid analysis of biological matrices can be made utilizing highly selective group separations, an attractive feature when many samples are to be processed.

TABLE 1. METHOD OF ANALYSIS: ION EXCHANGERS WITH NAA

	Concentrations in PPM		
SRM/SPL No.	Cu	As	Sb
Tomato - 1	10.8	.198	.109
Tomato - 2	11.3	.249	.147
Tomato - 3	11.0	.227	.084
Tomato - 4	11.3	.261	.128
Ave:	11.1 ± .2*	.234 ± .028*	.117 ± .027*
Pine Needles - 1	2.96	.195	.235
Pine Needles - 2	2.98	.193	.218
Pine Needles - 3	2.80	.223	.201
Pine Needles - 4	3.20	.185	.210
Ave:	2.98 ± .16*	.199 ± .016*	.216 ± .014*
Spinach - 1	10.8	.116	.049
Spinach - 2	10.8	.109	.040
Spinach - 3	10.6	.110	.043
Spinach - 4	11.0	.139	---
Ave:	10.8 ± .2*	.118 ± .014*	.043 ± .004*

*Errors are expressed as the standard deviation of the mean.

TABLE 2. A COMPARISON OF NAA RESULTS USING THE DESCRIBED SEPARATION PROCEDURE TO OTHER INDEPENDENT METHODS

Code	Element	Spinach	Pine Needles	Tomato Leaves
1	Cu	10.8 ± .2	2.98 ± .16	11.1 ± .2
4	Cu	12.7 ± .4	3.2 ± .4	11.5 ± .2
3	Cu	11.5	2.9	10.6
1	As	.12 ±.01	.20 ± .02	.23 ±.03
2	As	.17 ±.05	.21 ± .04	.29 ±.05
1	Sb	.043 ±.004	.22 ± .01	.12 ±.03

Codes: 1 - Neutron Activation Analysis with Chemical Separation.

2 - Colorimetry.

3 - Isotope Dilution Spark Source Mass Spectrometry.

4 - Atomic Absorption Spectrometry.

SECTION 5

THE CLEANING, ANALYSIS AND SELECTION OF CONTAINERS FOR TRACE ELEMENT SAMPLES

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INTRODUCTION

The determination of trace elements at very low levels, particularly in liquid samples, has been found to be biased by the analytical blank and can often be attributed in large part to contamination from sampling and storage containers (1,2,3,4). Synthetic polymeric materials are often employed as containers in the expectation that contamination will be less than if glass or metallic containers were used. The degree of improvement depends on the plastic and on the cleaning procedure (if any) employed to clean the plastic before use. The literature is contradictory on this topic (1). The present work examines several materials and cleaning methods in an effort to define a set of materials and procedures adequate for most work at the ng/g level.

For long term storage of liquid samples, an important consideration together with low contamination levels is a low rate of loss of water vapor. Factors to be considered include permeability, wall thickness, and closure integrity and are best determined by experiment. The rate of loss which can be tolerated is dependent upon both the accuracy desired and the amount of time during which the sample must be stored. For high accuracy work over a storage period of 10 years an annual rate of loss of 0.1 percent or less per year is necessary.

EXPERIMENTAL

A number of plastic materials were studied, including bottles constructed of conventional polyethylene (CPE), linear polyethylene (LPE), polypropylene (PP), polymethylpentane (PMP), polycarbonate (PC), polyvinylchloride (PVC), and several types of Teflon (TFE, FEP). In addition specimens of ethylenetetrafluoroethylene copolymer (Tefzel or ETFE), Teflon PFA, polystyrene (PS), and Teflon pipe sealing tape (TPT) were examined. The containers used for these studies were carefully selected to be free from visible occlusions and were average in wall thickness, weight, and other characteristics. The studies of these materials may comprise three types of experiments: a gravimetric study of the rate of loss of water from bottles, isotope dilution mass spectrometric (IDMS) analysis of the impurities leached from a bottle during a cleaning process, and neutron activation analysis (NAA) of the impurities contained within and leached from the plastics.

Gravimetric Studies. Two bottles each of CPE, PP, PVC, PMP, and PC were obtained from commercial sources (Bel Art, Nalge or equivalent). The bottles were 1 liter or 500 ml capacity, and except for rinsing with 95 percent ethanol followed by distilled water they were not subjected to a special cleaning procedure. All of the containers were weighed, then were filled to the same relative level with distilled water and re-weighed. The second container of each type was retained as a weighing tare to correct for the influence of changes in the barometric pressure, relative humidity and temperature, and loss of plasticizer. The samples were re-weighed after 17 days and 66 days to establish a rate of loss of moisture.

IDMS Studies. Leach solutions from one liter or 500 ml size bottles of FEP, CPE, LPE, and PC were examined by spark source IDMS (multi-element) and by thermal emission IDMS (for lead only). The general principles and techniques used have been given elsewhere (5,6). The bottles were rinsed with distilled water to remove any surface contamination and three sets of bottles were filled with a (1+1) mixture of ultra-pure HCl or ultra-pure HNO_3 and ultra-pure water (7). The bottles were allowed to stand at room temperature for one week, with the exception of the FEP bottle which was heated to 80 °C for one week.

Aliquots of the contents of one set of bottles leached by (1+1) HCl were spiked with ^{206}Pb. Aliquots from two other sets of bottles, leached by (1+1) HCl and (1+1) HNO_3 respectively, were spiked with a 19 element multi-spike (7) for the spark source mass spectrometer (SSMS). The spiked solutions were then wallevaporated to several drops under a Class 100 laminar flow clean hood. The sample solutions were analyzed in the SSMS (multi-element) or thermal source mass spectrometer (for Pb only).

The first set of bottles were then re-filled with a (1+1) mixture of ultra-pure HNO_3 and ultra-pure water and the entire cleaning, sampling, spiking, analysis sequence was repeated as before. Finally, the bottles were filled with high purity 0.5 percent (wt./wt.) HNO_3, sampled after one week and again after two months, and then the aliquots were spiked with ^{206}Pb and subsequently analyzed.

NAA Studies. Specimens of 0.2-2 grams (4-12 cm^2) were cut from new 0.5-1 ℓ plastic bottles (CPE, LPE, PP, PMP, PC, PVC, and FEP), sheet (TFE and PFA), bottle caps (ETFE), boxes (PS) or tape (TPT). The samples were pre-cleaned by rinsing lightly with 95 percent ethanol followed by distilled water. All irradiations were performed at a thermal neutron flux of 1.3×10^{13} n $cm^{-2}sec^{-1}$ and a gamma flux of 2×10^7 rad/hr (8). Other positions in the reactor offer higher neutron flux, but at the expense of a much higher gamma flux per neutron, and hence a much higher radiation damage to the samples.

NAA Experiment 1: The samples packaged in high purity LPE film (9) were irradiated for two minutes, the edges trimmed, and the induced radioactivity determined by counting on a Ge(Li) gamma detector. The samples were weighed, cleaned in (1+1) HCl by heating at 100 °C for two hours, rinsed with high purity water, dried, repackaged, and irradiated and counted again. After a similar two-hour leach in hot (1+1) HNO_3 the plastics were analyzed a third time.

NAA Experiment 2: Different samples of the same plastics were used in measurements of long-lived activation products. After irradiation (4-6 hrs) and decay of short-lived activities, the remaining radioactivation products were counted. The samples were then leached in (1+1) HCl at 100 °C, rinsed, and then leached in hot (1+1) HNO_3. Both leach solutions and the cleaned plastics were then counted on a gamma-ray detection system.

RESULTS AND DISCUSSION

Gravimetric Studies. The results are summarized in Table 1. Two of the bottles (PP and CPE) have water loss rates which are compatible with the long term storage of aqueous samples. In addition, data previously obtained by Moody, et al, (10) indicated an annual rate of water loss of ∿0.05 percent from one liter Teflon FEP bottles. PVC, PMP, and PC bottles may be adequate for storage over short time periods. Losses due to transpiration may be reduced by sealing the bottle in a commercially available vapor barrier, e.g., a polyethylene-coated film of aluminized mylar. The practice of using a desiccator partially filled with water to act as a humidity chamber to slow transpiration losses is recommended although one must be careful that high humidity conditions do not cause a weight gain (absorption of moisture).

None of the observed loss rates should be construed as permeability data since the closure may have been responsible for a significant portion of the total water loss. The results obtained in this work for CPE are similar to those observed by Curtis, et al, (11).

IDMS Studies. The results obtained for the leaching of lead from FEP, LPE, CPE, and PC containers are listed in Table 2. Lead was chosen as a model element for this study, partly because of the high accuracy obtainable by thermal source isotope dilution mass spectrometry and partly because lead is a common contaminant. The practice of cleaning bottles first with HCl and then with HNO_3 has been proven to be an effective procedure in our laboratories (7). Most of the cleaning of these containers is accomplished by only one week of soaking in HCl. The additional week of soaking in (1+1) HNO_3 removes additional lead only from FEP and PC. Patterson (2) has suggested that 0.5 percent HNO_3 is more efficient for the long term cleaning of Teflon containers from Pb contamination than is (1+1) HNO_3. In these experiments no further leaching of lead was detected after leaching with (1+1) HNO_3 except from the PC container. After one month of leaching with 0.5 percent HNO_3, no further lead was leached even from the PC container.

Data obtained by SSMS is compiled in Tables 3 and 4 for impurities leached by (1+1) HCl and (1+1) HNO_3 respectively. Again, the Teflon FEP bottles were heated to 80 °C while the others were leached at room temperature. The (1+1) HCl leached more than the (1+1) HNO_3 except for the FEP containers. A single element, Ca, is responsible for most of the difference between the (1+1) HCl and (1+1) HNO_3 in leaching Teflon FEP. Values which are below 2 ng/cm^2 or which are prefixed by $\leq$ are upper limits. That is, the concentration is below the optimum concentration range for the amount of spike isotope used or the concentration is near the blank or detection limit. The greater total quantity of various elements leached from FEP may be partially explained by the higher temperature (80° vs room temperature) used to clean the Teflon FEP container.

NAA Studies. The amount of impurities found in the uncleaned plastics as determined in NAA experiments 1 and 2 are summarized in Table 5. A comparison of the impurities found in CPE in this study shows agreement with that found by other workers (4,9,12-15) to approximately an order of magnitude. It is interesting to note that there has been no clear change in trace elemental impurities in over 25 years of manufacture (12). The ten materials studied show striking differences in trace element composition. The purest materials are TFE and CPE, in agreement with the experience of numerous workers (1). Polystyrene was also very clean. Many materials showed easily detectable amounts of a few elements. Some of these elements are residues of polymerization catalysts (16).

Interpretation of the acid-leaching experiments is complicated, particularly with the long irradiation in the second experiment, by the fact that the experiments are imperfect models of a reagent or analyte stored in a bottle. Those atoms of trace elements leached and detected in the leachate in the second experiment are precisely those not representative of undisturbed trace elements in an undisturbed matrix. The radioactive atoms underwent recoil on absorption of a neutron and thus may have been more labile than their inactive neighbors. In addition, the matrix suffered radiation damage from some 10^8 rads of gamma radiation and the halocarbons were damaged additionally by the absorption of nuclear recoil energy and beta radiation induced in major constituents of the matrix. However, detailed examination of the leaching results for sodium, the only element common to the two experiments, shows that radiation damage does not seem to greatly affect the general conclusions (Table 6) which are discussed below.

The results of the acid leaching experiments for all elements (Table 7) are compiled from the differences in trace element composition of the plastic before and after leaching in the first and second NAA experiments, and from direct measurement of leached tracer in the second, in a manner similar to the sodium results in Table 6.

With the exception of Na in pipe tape, (perhaps a special case because of its high surface/volume ratio), and to a lesser extent Na in CPE, the trace elements studied are in general not leached from the polymer matrix even with the rather severe acid treatment used here. It may be inferred that the bulk of most trace elements present is distributed throughout the matrix, and not merely on the surface.

The hydrochloric acid leach in experiment 1 increased the chloride concentration in all the plastics corresponding to a 10^{-4} cm thickness of 1+1 HCl in the surface of polypropylene, and less in the other materials. This inward diffusion of HCl is in accord with the water loss measurements, both experiments leading to a diffusion coefficient for aqueous solutions in polyolefin of on the order of $D=10^{-12}$ cm^2/sec. With this value of D, the rms diffusion length in one year is 0.1 mm.

CONCLUSIONS

Differences were observed between NAA and IDMS results for trace elements leached from the plastics. At least some of these differences may be in the way the samples were leached. For IDMS work the leaching was carried out within intact bottles. In contrast, the NAA studies examined the trace elements leached from both sides of a small sample of plastic immersed in acid. Since at least some containers are known to be blow molded, the higher results obtained by NAA may simply reflect a difference in contamination levels between inside and outside walls, the NAA data probably including contamination from the mold.

With the exception of Teflon FEP, (1+1) HCl has been found to be the better cleaning agent. However, HCl and HNO_3 appear to leach various elements with different efficiencies, thus, the use of both acids one after the other is recommended. The various Teflons and CPE bottles have been found to be the least contaminating bottles once they have been cleaned. Many methods have been suggested and several have been shown to be sufficient for the purpose at hand (1,2,7).

Suggested Method for Cleaning Bottles. From the present work, that of Karin et al. (15), and earlier work in this laboratory (3,7), a procedure is suggested which may be suitable for most contemporary trace element work. The procedure is outlined in Table 8. Only reagent grade acid is necessary for the acid leaching; but, ultrapure distilled water should be used for the final step.

ACKNOWLEDGEMENT

The authors wish to acknowledge the contributions of T. J. Murphy, P. J. Paulsen, and J. W. Gramlich for the IDMS analysis of solutions stored in plastic containers, and of D. A. Becker for numerous constructive suggestions.

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TABLE 1. ANNUAL RATE OF LOSS OF WATER FROM CONTAINER MATERIALS

Container	After 17 Days	After 66 Days
CPE	0.116%	0.109%
PP	0.034%	0.049%
PVC	0.429%	0.601%
PMP	0.988%	1.018%
PC	1.65%	2.00%

TABLE 2. LEAD LEACHED FROM CONTAINERS (ng/cm^2)
DETERMINED BY IDMS

Bottle	(1+1) HCl 1 week	(1+1) HNO_3 1 week	0.5% HNO_3 1 week	0.5% HNO_3 2 months
FEP	0.41	0.014	---	---
LPE	0.20	---	---	---
CPE	0.18	---	---	---
PC	0.36	0.023	0.023	---

--- no significant amount over blank level

TABLE 3. IMPURITIES LEACHED FROM PLASTIC CONTAINERS IN ONE WEEK BY (1+1) HCl (ng/cm^2) DETERMINED BY IDMS

Elements	Teflon FEP	LPE	CPE	PC
Pb	2	0.6	18	10
Tl	$\leq$1	$\leq$0.6	3	0.7
Ba	2	1	0.3	3
Te	2	---	0.7	---
Sn	1	$\leq$1	$\leq$0.8	13
Cd	0.6	0.2	0.2	$\leq$8
Ag	$\leq$6	---	---	---
Sr	$\leq$1	0.2	0.2	0.3
Se	0.8	0.4	$\leq$0.3	$\leq$0.5
Zn	4	9	1.0	---
Cu	6	1	0.7	$\leq$6
Ni	0.8	0.8	0.3	0.3
Fe	16	1	1.0	$\leq$49
Cr	4	0.8	0.3	$\leq$5
Ca	2	60	0.8	$\leq$16
K	1.6	1	0.7	$\leq$5
Mg	1.0	0.4	0.7	0.8
Al	4	4	10	3
Na	2	6	42	8
	Σ58	Σ89	Σ81	Σ130

TABLE 4. IMPURITIES LEACHED FROM PLASTIC CONTAINERS IN ONE WEEK BY (1+1) HNO_3 (ng/cm^2) DETERMINED BY IDMS

Elements	Teflon FEP	LPE	CPE	PC
Pb	2	2	0.7	0.3
Tl	$\leq$1	$\leq$1	1	$\leq$0.8
Ba	4	$\leq$0.2	<2	0.3
Te	0.6	0.2	$\leq$0.5	0.3
Sn	1	1	$\leq$0.8	0.2
Cd	0.4	0.2	0.2	0.3
Ag	$\leq$8	0.2	---	---
Sr	0.2	1	0.2	$\leq$0.2
Se	0.2	0.4	3	0.5
Zn	4	8	2	0.8
Cu	2	0.4	2	0.8
Ni	2	1.6	0.5	0.7
Fe	20	3	3	3
Cr	0.8	0.2	0.8	0.3
Ca	80	0.6	10	3
K	2	2	2	2
Mg	8	0.6	0.7	2
Al	6	1	1	5
Na	6	10	8	3
	Σ148	Σ50	Σ38	Σ23

TABLE 5. CONCENTRATIONS OF TRACE ELEMENTS IN PLASTICS DETERMINED BY (ng/g)

	CPE	LPE	PP	PMP	PS	PC	PVC	TFE	FEP	PFA	TPT	ETFE
Na	1.3E3	15E3	4.8E3	200	2.2E3	2.7E3	20E3	160	400	100	2.3E3	600
Al	500	30E3	55E3	6.2E3	500	3.0E3		230	200		29E3	
Cl	7E3	30E3	180E3			50E3	major		800	50E3	7E3	1E6
K	>5E3	>600		>200					90E3		3E3	1.1E3
Ca		800E3										
Ti		5E3	60E3	5E3	1E3						2E6	
Mn		10	20	10	20				60	20	20	
Co			40			6				90		
Zn		520E3		33E3							14E3	
Br	>20	800	>5	>2	>1	29E3	>6	>2		16E3		240
Sn							2.4E6					
Sb	5	200	600									
Lz					0.3			0.6				1
W									700			
Au			0.1	0.6	0.04	0.03		0.4			0.2	0.4

Notation: 1.3E3=1.3x10^3 ng/g. When lower limits are quoted, the element sought was short-lived and found only in the leachate or leached sample. Hence the concentration in the unleached plastic may have been greater but was not visible in the presence of large quantities of short-lived interfering radioactivities. Blanks in the table represent the extreme where the element sought for was not visible. Detection limits may be roughly judged from values in adjacent columns.

TABLE 6. REMOVAL OF SODIUM FROM PLASTICS

	Expt. 1 Short Irradiation			Expt. 2 Long Irradiation			
Matrix	Before Cleaning	Post HCl	Post HCl + HNO_3	Before Cleaning	HCl Leach Liquid	HNO_3 Leach Liquid	Post Cleaning
CPE	600 ±100			1290 ±30	1480 ±10	2.0 ±0.3	330 ±20
LPE	17000 ±3000	15000 ±3000	14000 ±1000	13200 ±200	273 ±2	5.9 ±0.5	13200 ±100
PP	5000 ±1000			4640 ±70	480 ±3	32 ±1	4330 ±60
PMP	220 ±50			160 ±5	52 ±1	1.0 ±0.1	120 ±10
PS	2000 ±400	1500 ±400		2450 ±40	60 ±1	9 ±1	2430 ±70
PC	2400 ±400			2860 ±50	68 ±1	4 ±1	2850 ±100
PVC				19500 ±400	190 ±10	28 ±3	21000 ±900
TFE	170 ±40			160 ±10	111 ±2		
FEP				400 ±20	242 ±3		
PFA	180 ±50			39 4	15 ±1		10 ±10
TPT	2900 ±600		1900 ±200	2270 ±50	1890 ±20	144 ±3	380 ±40
ETFE				575 ±15	427 ±3	1.2 ±0.1	208 ±3

Concentrations are in ng/g plastic. Errors quoted reflect counting statistics only, and so underestimate the total error of measurement. Note that the two "leach liquid" columns give directly the amount of Na removed by the leach step; all other columns give the amount present in the plastic sample. Blanks imply that Na was not detected.

TABLE 7. SEQUENTIAL LEACHING OF TRACE ELEMENTS FROM PLASTICS (ng/cm^2)

	CPE	LPE	PP	PMP	PS	PC	PVC	TFE	FEP	PFA	TPT	ETFE
Na	200+0.4	70+1	100+7	20+0.4	20+8	20+1	30+10	80+	60+	20+	50+4	300+1
Al	50+											
K	1000+	100+		80+					90+			400+
Co						+2						
Zn		50+30									20+	
Br	3+	2+0.5	1+	1+	0.3+0.1	7+20	2+	10+				
Sb	1+	3+										
La					0.1+			0.4+				0.7+
W									4+0.2			
Au			0.03+0.01		0.01+	0.01+		0.3+			0.01+	0.3+0.01

Determined by NAA. The two numbers given (in ng/cm^2) are respectively the amount leached by 1:1 HCl and by 1:1 HNO_3 in 2 hours at 100°C. A blank means that the constituent was not detected. See also footnote to Table 5.

TABLE 8. SUGGESTED METHOD FOR CLEANING PLASTIC CONTAINERS

1. Fill with 1+1 HCl (AR grade).
2. Allow to stand one week at room temperature (80° for Teflon).
3. Empty and rinse, with distilled water.
4. Fill with 1+1 HNO_3 (AR grade).
5. Allow to stand one week at room temperature (80° for Teflon).
6. Empty and rinse with distilled water.
7. Fill with purest available distilled water.
8. Allow to stand several weeks or until needed, changing water periodically to assure continued cleaning.
9. Rinse with purest water and allow to dry in a particle- and fume-free environment.

SECTION 6

EVALUATION BY ACTIVATION ANALYSIS OF ELEMENTAL RETENTIONS IN BIOLOGICAL SAMPLES AFTER LOW TEMPERATURE ASHING

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INTRODUCTION

Low temperature ashing (LTA) is the low temperature (~100 °C) decomposition of organic or biological samples with atomic oxygen produced by an electrodeless radio-frequency (RF) discharge. There are a number of advantages in using LTA as the first step in an analytical method whether by nuclear activation or otherwise:

1. Since the oxidation temperature is low, volatility losses are minimized during the destruction of large quantities of organic material.

2. There are no electrodes to contaminate the sample.

3. A sample can be more readily dissolved after LTA than by alternative methods. Wet ashing techniques with hot oxidizing acids or strong alkalis are potentially hazardous, sometimes incomplete, and usually susceptible to contamination because of the large amounts of reagents required.

4. High temperature ashing in a muffle furnace introduces the possibility of contamination from containers or the furnace walls as well as loss of some elements by volatilization. The convenience of the LTA method can be demonstrated by reference to a publication concerning the spectrophotometric determination of boron extracted from ashed animal tissues (1). Four grams of freeze-dried tissue were ashed 40-50 hours at 100-150 watt forward RF power. After the LTA treatment, the sample could be dissolved in 2 ml of 1N HCl.

5. In the X-ray fluorescence method, which is strongly matrix dependent, LTA reduces the sample to a silicate-carbonate-oxide form, which minimizes errors arising from matrix interferences.

6. In spark-source mass and emission spectrometry the ashed residue, when mixed with powdered graphite, gives a uniform consistency which also reduces matrix error.

With specific respect to activation analysis, one can identify several advantages of beginning the analysis of a biological sample with freeze-drying followed by LTA.

1. A very high neutron flux can badly char a biological or organic sample leaving it with an undesirable consistency. That the LTA method is very efficient for removing organic carbon is demonstrated by an American Society for Testing and Materials procedure (2) which specifies the technique

in removal and hence determination of organic carbon as opposed to carbonate in sediment samples.

2. LTA results in a substantial reduction in the volume of the sample. This can yield a corresponding reduction in electron accelerator or neutron generator irradiation time in photon or 14-MeV neutron activation analysis.

3. Since lengthy wet digestions are eliminated, a sample after irradiation can be dissolved more quickly and hence short-lived nuclides can be more quickly separated from the sample for counting.

4. In the instrumental photon activation analysis of samples containing large amounts of carbon, it is usually necessary to delay counting for a few hours after irradiation to allow the ^{11}C produced by the reaction $^{12}C(\gamma, n)^{11}C$ to decay. Some shorter-lived nuclides are therefore not detected. Many of them could be detected by removal of the carbon prior to irradiating.

The possibility exists in the LTA method of the loss of elements of interest during the ashing process. Studies to evaluate elemental retention during ashing have been conducted with radioactive tracers (3). This is not an entirely satisfactory procedure since an element may not be present in a biological sample in the same chemical form as was used in the tracer studies. This paper describes a study, using activation analysis, of loss or contamination of trace elements during LTA. It is hoped that this will be useful not only in analytical chemistry for the reasons listed above, but also to evaluate LTA as a potential processing method for the retention of biological samples by the National Environmental Sample Bank over several decades of time for purposes of baseline studies to evaluate increases or decreases in pollution.

EXPERIMENTAL

A commercial model LTA apparatus capable of 100 watts forward power was used in these experiments. A schematic of the apparatus is shown in figure 1. The sample was placed in a borosilicate glass ashing chamber which was briefly rotated by hand to induce the organic sample to spread as evenly as possible on the inside surface. The cold trap was filled with crushed dry ice (liquid nitrogen would condense explosive ozone). The system was pumped down to 1.3-2.6 Pa (10-20 millitorr). The oxygen flow rate was adjusted so as to maintain a pressure of 13-40 Pa (100-300 millitorr). Forward power was usually set to the maximum of 100 watts. Tuning to optimum was accomplished by observing the maximum intensity in discharge glow in the tube.

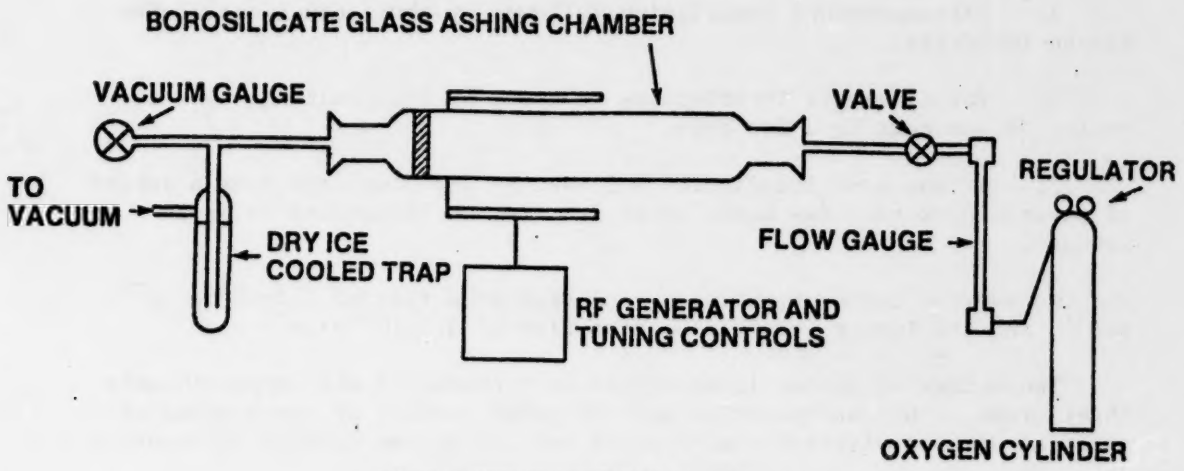

Figure 1: Block Diagram of LTA Apparatus

Preliminary experiments involving addition of tracers to biological materials and determining retention of each isotope after ashing verified previously published data /3/ and served to establish and perfect the basic ashing technique. After these experiments, it was decided to study three NBS Standard Reference Materials in detail; 1571-Orchard Leaves, 1577-Bovine Liver, and 1632-Trace Elements in Coal.

Samples of about one gram were ashed for a period of several hours. It was impossible to remove all of the ashed material from the tube, thus it was necessary to determine the exact amount of material removed. This was first attempted by weighing of the tube which proved difficult, since the only balance capable of accommodating the 200 g in weight, 30 cm in length ashing tube was accurate to only + 2 mg. A second and more successful technique involved the use of a nonvolatile radioactive tracer that wouldn't

interfere with later activation analysis. The uniform distribution of the tracer throughout the bulk sample was demonstrated by taking several small samples from the bottle and measuring their specific activities. Subsequently, it was found possible to use the activation product of an element demonstrated to be nonvolatile as an internal standard for this purpose.

The relative amounts of the elements in ashed and unashed samples were determined by nondestructive neutron activation analysis with the NBS 10 megawatt nuclear reactor and photon activation analysis with the NBS electron linear accelerator.

For the reactor irradiation a paired experiment was carried out using approximately one gram of unashed material compared to the ashed residue of one gram of material. Three series of neutron irradiations were conducted:

1. Fifteen-second irradiation followed by three countings at few minute intervals,

2. Twenty-minute irradiations followed by 2-3 countings over a period of one hour to a few days,

3. A four-hour irradiation followed by 2-3 countings over a period of several days to a few weeks after irradiation (depending on Na-24 levels).

The two shorter irradiations were conducted at a flux of 1.3×10^{13} n·cm^{-2}-sec^{-1}, and the longer irradiation at a flux of 5.6×10^{13} n·cm^{-2}-sec^{-1}.

Two series of photon irradiations were conducted with approximately three grams of unashed material and the ashed residue of three grams of material with an electron energy of 35 MeV and a beam current of about 50 μA.

1. Thirty to sixty minute irradiation followed by 2-3 countings over a period of two hours to a few days after irradiation,

2. Two to six hour irradiation followed by 2-4 countings over a period of two days to a few weeks after irradiation.

Irradiated samples were counted with a 75 cc Ge(Li) detector and a 4096 channel analyzer using one-half of the memory. Molybdenum, iodine, and zinc by photon activation analysis were also determined with a low energy photon detector (LEPD). Photopeak counting rates were corrected for times of irradiation and decay and ratio of weights of unashed material and ashed residue corrected for weight loss.

RESULTS AND DISCUSSION

The experimental study of the observation of ashing losses was carried out in three phases. The first consisted of spiking samples of interest with radioactive tracers of known chemical states. This phase was simple to conduct as the isotopes could be so added to avoid interference in radioactive counting. This first phase served to assist in development of handling techniques for subsequent work. The results with radioactive tracers showed the retention of zinc, cadmium, arsenic, palladium, rhenium, antimony, iron, platinum, iridium, gold, and silver. A previous paper (3) had reported the loss of gold and silver but this phenomenon was not confirmed here. Mercury and osmium were lost after several hours of ashing in every case.

The second phase consisted of irradiating portions of the three samples, counting them, then ashing and recounting. These experiments added to the retention list the following elements: sodium, potassium, rare earths, and selenium.

The third phase, which was the major part of this study, involved the simultaneous activation, either by thermal neutrons or high-energy photons, of an untreated portion of the samples and an ashed portion. This study thus established loss data on elements in the chemical form they would actually be found in three representative matrices. Table 1 gives typical weight losses and carbon losses as measured by the photonuclear reaction $^{11}C(\gamma,\alpha n)^{7}Be$ for an ashing period of approximately 12 hours and power of approximately 70 watts. In addition to carbon, the loss of chlorine, bromine, and iodine was observed in all three matrices as well as the expected loss of mercury. Table 2 gives experimental recoveries of retained elements.

TABLE 1. TYPICAL WEIGHT AND CARBON LOSSES FOR ASHING PERIODS OF 15 HOURS AND RF POWER OF 70 WATTS

	% Weight Lost	% Carbon Lost
Coal	80	75
Beef Liver	90	98
Orchard Leaves	75	80

Figure 2 shows, in the format of the periodic chart, the elements detected and not lost during ashing in all experiments. The symbols in the individual square of each element, OL, C, and BL stand for orchard leaves, coal, and beef liver, respectively, in which the element was determined. In addition, some elements reported retained in the literature determined by neutron activation analysis (4), atomic absorption spectrometry, (5) or spectrophotometry (1) are included. The numbers refer to references.

H																HE
LI	BE											B 1	C	N	O	F	NE
NA C OL BL	MG C OL BL											AL C OL BL	SI	P	S	CL	AR
K C OL	CA C OL BL	SC C OL BL	TI C OL	V C	CR C OL BL	MN C OL BL	FE C OL BL	CO C OL BL	NI C OL	CU 5	ZN C OL BL	GA C	GE	AS C OL	SE C OL BL	BR	KR
RB C OL BL	SR C OL	Y	ZR C OL	NB	MO OL BL	TC	RU	RH	PD	AG 4	CD 5	IN	SN	SB C OL BL	TE	I	XE
CS C OL BL	BA C OL BL	LA C OL BL	HF C OL	TA C	W C	RE	OS	IR	PT	AU	HG	TL 5	PB C OL	BI	PO	AT	RN
FR	RA	AC															

CE C OL BL	PR	ND	PM	SM	EU C OL BL	GD	TB	DY	HO	ER	TM	YB	LU
TH C OL BL	PA	U	NP	PU	AM	CM	BK	CF	ES	FM	MD	NO	LW

1. J.W. Mair, Jr., and H.G. Day, Anal. Chem. 44 (1972) 2015. Matrix was animal tissue.
4. D.Behne and P.A. Matamba, Z. Anal. Chem. 274 (1975) 195. Matrix was blood serum.
5. B.B. Stafford, Proceedings 2nd Conf. Trace Subs. in Environmental Health (1968) Univ. of Mo. Matrix was atmospheric particulate.

Figure 2: Elements Retained During LTA

Although some of the results have been previously reported, we include data here on many additional elements, including those particularly susceptible to loss such as arsenic, selenium, and chromium, since they are known to form compounds which are volatile at the temperatures of LTA.

TABLE 2. PERCENT OF ELEMENT RETAINED (EXPERIMENTAL)

	Coal	Orchard Leaves	Beef Liver
Sodium	98	100	104
Magnesium	98	101	95
Aluminum	105	-	112
Potassium	101	106	-
Calcium	99	101	99
Scandium	100	101	-
Titanium	105	99	-
Vanadium	98	-	-
Chromium	99	106	94
Manganese	102	102	105
Iron	96	102	99
Cobalt	108	95	106
Nickel	99	99	-
Zinc	107	102	100
Gallium	91	-	-
Arsenic	110	92	-
Selenium	99	105	-
Rubidium	98	98	92
Strontium	98	106	-
Zirconium	104	108	-
Molybdenum	93	94	-
Antimony	104	108	-
Cesium	102	104	-
Barium	105	109	-
Lanthanum	107	95	95
Cerium	99	98	100
Europium	98	-	94
Hafnium	96	98	-
Tantalum	109	-	-
Tungsten	97	-	-
Lead	106	97	-
Thorium	103	92	-

It is also significant to note that, equal in importance to loss of trace elements, there was no pick-up of contamination during the ashing process as would be evidenced by an increase in the amount of the element after ashing. This is in spite of the fact that only very ordinary precautions were taken to guard against contamination during cleaning of the borosilicate glass ashing tube with ordinary laboratory detergent followed by rising with water, hot dilute hydrochloric acid and finally deionized water and drying in an oven at 100 °C.

An application of immediate interest is those elements which upon thermal neutron or photon activation yield radioisotopes with half-lives in the range of 2-30 minutes and are not detectable in the typical biological sample without separation. Since after ashing, biological samples, especially animal tissue, are rapidly dissolved in dilute mineral acid, one may consider radiochemical separations in the determination of magnesium, aluminum, titanium, and vanadium in the case of thermal neutron activation and potassium and iron in photon activation.

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SECTION 7

STABILITY OF ELEMENTAL COMPONENTS IN MULTI-TRACE ELEMENT WATER STANDARD

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INTRODUCTION

The study of natural water samples which require trace element compositional data poses a specially difficult analytical problem (1,2). The extremely low concentrations of many toxic elements lead to all the normal problems associated with ultratrace analysis. These problems are compounded by the chemical mobility of the species being determined (3). Also, unique problems are encountered in proper sampling and storage of samples for laboratory analysis (4,5). The subject of valid sampling of natural waters will not be discussed here although it must be emphasized that improper sampling can invalidate any study of these waters.

Most analytical methods used for trace element analyses are relative techniques; that is, the signals developed must be compared to those obtained from standards of known compositions. Unfortunately, experience has shown that pure, single element aqueous standards do not compensate for interferences or changes in signal response caused by the analyte matrix. In an effort to provide an adequate standard for trace metals analysis in natural waters, the National Bureau of Standards is currently certifying a mixed trace element in water Standard Reference Material (SRM).

Two important considerations in the development of any standard are the homogeneity of and stability of the certified species. In the case of water standards, elemental homogeneity is easily achieved by careful mixing of the bulk solution using a single large container. Long-term stability, however, is a recognized problem for many elements at low concentrations. Mineral acids have been employed as stabilizing agents and are usually successful in the ppm range and above (1,6,7). However, in the ppb concentration range, methods for long-term storage have not been documented.

Two studies have been conducted to verify the long-term stability of ten trace elements of wide interest to water chemists. The first study was conducted with radioactive tracers and was designed to indicate any elemental losses to container walls. This study was continued for 255 days and indicated acceptable stability for all elements included. Following the initial study, a more comprehensive investigation was carried out using simultaneous analyses by atomic absorption, neutron activation, and isotope dilution spark source mass spectrometry. This second study was designed to indicate any problems of concentration elevation due to reagent blanks and leaching of trace elements from contact materials. The results of this investigation indicated that commercial grade polyethylene or Teflon containers were equally acceptable

except for the element mercury. In the case of mercury, polyethylene containers are very much more susceptible to contamination from external mercury than are Teflon containers, because of higher permeability of mercury through polyethylene.

The results of the stability studies indicated that the trace elements of interest could be stabilized for more than one year using 0.5 Molar HNO_3. With this assurance, a multitrace element water standard containing twenty trace elements has been prepared.

EXPERIMENTAL

Initial stability studies were carried out using radiotracers of five important elements, As, Se, Hg, Cd and Zn. A master solution was prepared containing approximately 50 parts-per-billion (1 ppb = 10^{-9} g/g) of the elements of interest. The solution was stabilized with HNO_3 at a final concentration of 0.5 M HNO_3 and gold ion at a concentration of 10^{-8} g Au/g water. The gold ion was added to stabilize mercury as described previously (8). The final solution was stored in a one liter Teflon bottle under normal laboratory conditions.

Concentration stability was determined by withdrawing 1 ml of the standard solution and counting the solution at a fixed geometry with a Ge(Li) detector coupled to 4096 channel pulse height analyzer. Individual photopeak activities were quantified by the total peak area method and decay corrected to the beginning of the experiment. Relative elemental concentrations were obtained from the counting data for a period of 255 days. At this time, the errors in the decay corrections of the ^{73}As and the ^{203}Hg became large and the experiment was terminated.

The tracer experiment gave information as to possible elemental loss phenomenon. However, that type of experiment yielded no information on potential blank problems, either from preparatory reagents or from the storage containers. Thus, a concurrent study was conducted to provide information on these problems. In this study, a trial solution of a multiple element standard was prepared under clean room conditions and analyzed over a 17-week period. The trial solution was stored in a precleaned one liter Teflon and polyethylene bottles. The bottles were cleaned and tested for blank using a previously described procedure (8).

To make the trial solution and also the final Standard Reference Material, concentrated solutions of individual elements were prepared by dissolving weighed quantities of spectrographically pure metals or compounds of the elements of interest. By accurate dilution of these standard solutions, a test solution containing 18 trace elements plus mercury, gold and the four matrix elements Na, K, Ca, and Mg was made. In certain instances, most notably for Be, it was necessary to elevate the concentration over the level normally found in fresh waters in order to assure a reasonable chance for analysis by two independent methods. Analysis of this preliminary sample by NAA and AAS indicated no potential problems at these concentration levels.

For the final solution a single 55 gallon polyethylene drum and stirring paddle was cleaned in a manner similar to the storage bottles. Carefully weighed amounts of specially purified water and HNO_3 (2) were added to the 55 gallon drum with stirring. Three master solutions which together contained all 24 elements were carefully prepared by weight dilution of the original concentrated standard solutions. Aliquots of these three dilute master solutions were then added to the water and HNO_3 in the 55 gallon drum with constant stirring.

The resulting solution was stirred for several days and then to ensure mixing vertically throughout the drum, aliquots were removed from a spigot at the bottom and poured back into the top of the drum. Finally, after complete mixing, the solution was transferred into 230 precleaned one liter polyethylene bottles which were serially numbered. All of the dissolutions, dilutions, and other manipulations were carried out in a Class 100 clean laboratory using techniques designed to avoid contamination of the samples. Selected samples were taken for certification analyses and long-range stability studies.

RESULTS AND DISCUSSION

The initial stability study using radioactive tracers was continued for a period of 255 days. In this study, the initial specific activity of each isotope on day zero was defined as unit concentration, i.e., 1.000 relative units. Changes in concentration were then determined by a simple ratio of the specific activity at any time t, compared to that at time zero. Initial data were taken at frequent time intervals to detect any rapid changes in concentration. When no discernable changes were noted, the time interval between data points was prolonged. After 250 days, the errors introduced by the half life correction of data became unacceptably large for the shorter half lifed isotopes, thus the experiment was terminated.

With completion of this experiment, the data set for each element was analyzed by unweighted linear regression. Predicted final concentrations and slopes were calculated with associated errors. The individual data, predicted initial and final concentrations and slopes are given in Table 1. Two hypotheses were tested to indicate elemental stability. First, was to test equivalence between initial and final concentrations. In the case of the predicted initial and final concentrations, the differences were equal to or less than the standard deviations of this prediction, except in the case of Zinc. Even in that case, the difference was small. The second test was that of a nonzero slope for individual data sets. Again, only in the case of Zinc was a significant nonzero slope observed and that was again marginal.

In the case of Zinc, the positive slope gave a predicted final concentration which was slightly larger than the initial concentration. However, a real increase in zinc-65 isotopic concentration is a physical impossibility. The observed change is best attributed to either a small error in half life correction of the zinc-65 data or simply a statistical fluctuation.

Table 1. Trace element loss study using radioisotopes

Time in days	Isotopic concentrations relative to day zero				
	^{73}As	^{75}Se	^{203}Hg	^{109}Cd	^{65}Zn
0	1.000	1.000	1.000	1.000	1.000
9	1.006	1.012	0.999	0.997	1.012
14	1.041	1.041	1.026	---	1.032
22	---	0.991	1.031	0.965	1.033
35	0.997	1.007	1.008	0.976	1.036
79	1.027	1.000	0.983	0.938	1.012
94	1.080	1.049	0.997	0.973	1.031
255	0.977	0.981	1.032	0.984	1.057
Predicted Con. Day 0	1.025	1.016	1.006	0.978	1.017
Std. Dev. of Con. at Day 0	±0.017	±0.011	±0.009	±0.011	±0.006
Predicted Con. Day 255	1.001	0.992	1.021	0.971	1.055
Std.Dev. of Predicted Con.	±0.034	±0.022	±0.017	±0.021	±0.013
Slope	-0.000096	-0.00009	+0.00006	-0.00003	+0.00015
Std. Dev. of Slope	±0.00017	±0.00011	±0.00008	±0.00010	±0.00006
P(F)	.59	.42	.50	.79	.052

(P(F) significant if <0.05).

The tracer experiment indicated that the five elements studied could be stabilized at trace concentrations in water for at least one year.

A second study was initiated to provide information on possible blank problems as well as additional information on stability. In this study, a trial solution of a multiple element standard was prepared under clean room conditions and analyzed twice over 17-week period. The analytical results were obtained by three independent analytical methods, neutron activation (NAA), isotope dilution spark source mass spectrometry (ID-SSMS) and by atomic absorption spectroscopy (AAS). The individual methods used where those developed for SRM certification over the past ten years. The data obtained by the analyses were compared with elemental concentration obtained by calculation from dilution of the master spike solution. Data were also compared among analytical techniques for consistency. The results of the analytical study are given in Table 2. The analytical data presented are mean values obtained on a replicate set of two to four analyses by a given technique. Error levels are roughly indicated by the number of significant figures presented but

insufficient data were obtained to statistically estimate analytical errors. The comparison of data among techniques is a valid indicator of changes in elemental concentrations.

Table 2. Trace element stability study using independent analyses

Element	Calculated Concentration	Analytical[+] Technique	Initial Concentration	17-Week Concentration
			Concentration in µg/g	
Zn	4.89	NAA	4.96	5.08
		AAS	4.96	5.08
		SSMS	5.16	---
Cu	0.807	NAA	0.814	0.849
		AAS	0.851	0.856
		IDMS	0.845	0.848
As	0.0937	NAA	0.0603	0.0630
		AAS	0.064	0.060
Pb	0.0469	AAS	0.0512	0.0459
		IDMS	0.0468	0.0465
Mn	0.0418	NAA	0.0412	0.0470
		AAS	0.0400	0.0455
Cr	0.0473	AAS	0.051	0.053
		SSMS	0.0471	0.0465
Be	0.0397	AAS	0.0416	0.0416
		Spectro.	0.0406	0.0409
Cd	0.0137	NAA	0.0146	---
		AAS	0.0150	0.0149
		SSMS	0.0185	0.0145
Se	0.0101	NAA	0.0105	---
		AAS	0.0112	0.011
		SSMS	0.0098	0.0118
Hg	0.00184	NAA	0.0018	0.0019
		AAS	0.0018	0.0017

\+ NAA = Neutron Activation Analysis
AAS = Atomic Absorption Spectrometry
SSMS = Isotope Dilution Spark Source Mass Spectrometry
IDMS = Isotope Dilution Thermal Source Mass Spectrometry
Spectro = Spectrofluorimetry

The results of the analytical study were consistent with those of the tracer study. No indication of trace element loss was observed during the 17-week period. However, two interesting effects did occur. First it is obvious from the data that for arsenic, the calculated concentration was grossly different from the measured concentrations. There are two possible causes for this observed occurrence. First, it is possible that a large, rapid decrease in arsenic concentration did occur either by precipitation of the arsenic or by wall adsorption. However, it is difficult to imagine such a mechanism for an immediate large loss of arsenic which would not continue to be observed for a longer period of time. No such continued change in concentration was observed in the 17-week analysis. A more likely explanation is that the calculated concentration was in error due either to a mistake in weighing or volumetric dilution.

A second, and more subtle effect was observed for manganese where a slight increase in concentration over the 17-week period was observed. This increase was noted both by neutron activation and by atomic absorption. The effect is small, however, and subsequent analyses have not indicated a continued increase in manganese concentration. It is most probable that a small amount of manganese was initially leached out of the containers, even though those containers had been subjected to rigorous cleaning. The manganese concentration has not been observed to increase significantly in subsequent analyses. The element manganese will be carefully checked for an extended period of time in the certified Standard Reference Material.

The third observation from the analytical study was that some analytical procedures were in error. Even though the procedures used were considered to be the best available, more analytical research was needed to accurately analyze all elements at the concentrations selected for the standard. This problem is particularly evident with the cadmium data as determined by isotope dilution spark source mass spectrometry and with the copper data as determined by neutron activation. Research into the respective analytical methodologies was conducted and significant improvement was noted in the 17-week analyses.

The two studies on trace element stability indicate that a mixed trace element in water standard can be stabilized with 0.5 M HNO_3 in conjunction with trace gold ion. The studies illustrate the type of investigations which must be carried out before trace solution standards can be assumed to give valid data. A final solution has now been prepared, bottled, and analyzed for the certification of SRM 1643 Trace Elements in Water.

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